PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/04, 1/16, 1/18, G01N 21/64, 21/76 | A1

(11) International Publication Number: WO 96/28570

(43) International Publication Date: 19 September 1996 (19.09.96)

(21) International Application Number: PCT/US96/03223

(22) International Filing Date: 8 March 1996 (08.03.96)

(30) Priority Data:
08/402,995 9 March 1995 (09.03.95) US

(71) Applicant (*for all designated States except US*): XECHEM, INC. [US/US]; 100 Jersey Avenue, Building B, Suite 310, New Brunswick, NJ (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): PANDEY, Ramesh, C. [US/US]; 30 South Adelaide Avenue, Highland Park, NJ 08904 (US). NATHAN, Swami [US/US]; 24 Bay Berry Close, Piscataway, NJ 08854 (US). MISHRA, Jasleen [US/US]; 919 Sunrise Knoll Way, Houston, TX 77062 (US). MISHRA, Saroj, K. [US/US]; 919 Sunrise Knoll Way, Houston, TX 77062 (US).

(74) Agent: CRONK, Peter, J.; Duane, Morris & Heckscher, One Liberty Place, Philadelphia, PA 19103-7396 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A RAPID METHOD OF AND DIAGNOSTIC KIT FOR THE DETECTION OF MICROORGANISMS

(57) Abstract

A method and diagnostic kit for rapidly detecting the presence of a microorganism in any given sample is provided. More particularly, a method and diagnostic kit for rapidly detecting the presence of a microorganism in a urine sample for diagnosing urinary tract infection is provided. Methods and kits for determining antibiotic sensitivity, total cell count and the identification of a microorganism in a given sample are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia
AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

A RAPID METHOD OF AND DIAGNOSTIC KIT FOR THE DETECTION OF MICROORGANISMS

FIELD OF THE INVENTION

The present invention, in general, relates to the detection of the presence of a microorganism in a sample, and more particularly to the detection of the presence of a microorganism in a urine sample as an aid in the diagnosis and treatment of urinary tract infection.

BACKGROUND OF THE INVENTION

Urinary tract infection (UTI) is a significant major cause of morbidity in humans. The direct economic impact of UTI in terms of the cost of diagnosis and therapy and the indirect cost of lost work hours is immense. Nearly 100% of both females and males over age 60 suffer from UTI at least once in their lifetime. As many as 20% of all hospitalized patients who receive short-term catheterization develop a UTI. Consequently, UTI is the most common nosocomial infection in the United States. UTI is also a frequent complication in chronic stress syndrome, and can be associated with travellers' diarrhea, especially in females.

Other predisposing factors include poor personal hygiene, hormonal imbalance, urethral trauma, and unprotected sex. If untreated, the infection can lead to serious complications including, but not limited to, kidney damage, bacteremia, and death. The course of infection can be chronic or acute, and the latter form can progress very rapidly reaching the serious stage within a few hours.

UTI can be broadly classified into two categories: cystitis, which is an infection of the bladder, and pyelonephritis, which is an infection of the renal parenchyma. Another form of UTI which has only recently been defined is the acute urethral syndrome, mostly observed in sexually active women of child-bearing age.

UTI is predominantly caused by Enterobacteriaceae, which are normal intestinal flora. *E. coli* has been implicated in 70 to 80% of UTI in apparently healthy or ambulatory patients. However, the predominant etiologic agents of UTI in hospitalized patients and individuals repeatedly treated with antibiotics are the species of *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and Enterococcus faecalis*. Other organisms implicated include *Citrobacter freundii, Serratia spp.*, yeasts, *Proteus morganii*, and *Staphylococcus aureus*. Gram positive bacteria are relatively less frequently associated with UTI. The coagulase negative staphylococci, especially *Staphylococcus saprophyticus*, is probably the leading etiologic agent in this category.

UTI due to *Candida albicans* and other yeast-like fungi is commonly observed in diabetic women and patients on long-term antibiotics. *Chlamydia trachomatis* alone or in association with Enterobacteriaceae is the common cause of acute urethral syndrome. Unlike bacterial UTI, the infection due to yeast and chlamydia progress somewhat more slowly. For a more detailed discussion of this subject, see Chapter 18: Microorganisms Encountered in the Urinary Tract in Bailey & Scott's Diagnostic Microbiology (C.V. Mosby Co., 1990, 8th edition), which is incorporated herein by reference.

The currently available diagnostic procedures depend on the cultural examination of aseptically collected urine samples. Typically, isolation of 10^5 bacteria from a milliliter of urine is considered diagnostic of UTI. This mandates that the specimen be processed as soon as possible. Prolonged storage, even at low temperature, or

shipment to a diagnostic laboratory may adversely affect the diagnostic value of the culture examination. Thus, the ideal diagnostic services to UTI patients are mostly available if they are hospitalized or live close to a well-equipped modern microbiology laboratory.

Currently available diagnostic methods are not readily available, and the time required to determine even a tentative diagnosis is typically greater than 24 hours. Such a length of time is detrimental to both patients and physicians. Either the physician begins an empiric therapy, which may or may not be effective, or the patient chooses to ignore the symptoms, with potential aggravation into serious life-threatening complications. In any event, at least 24 hours are typically required before a provisional cultural result can be obtained. Species identification and antibiotic sensitivity determinations may require an additional 24 hours. In acute cases with a rapid course of infection, such a delay can be seriously detrimental to the well-being of the patient. In view of the rising number of cases due to multiple drug resistant bacteria, the common practice of treating empirically or without drug-sensitivity results is highly risky and undesirable.

Therefore, currently available methods, under ideal circumstances, require 24 to 48 hours before definitive results can be obtained. This length of time can result in serious or dire consequences for the patient. Even with respect to routine clinical infections, it is desirable to obtain a more rapid diagnosis than is currently available.

Furthermore, conventional methods are not suitable for rapidly screening or detecting the presence of a microorganism in a sample such as water, beverages and food.

Nor do conventional methods rapidly detect the presence of a microorganism in other biological fluids, in addition to urine.

There therefore exists an urgent need for a rapid method of detecting the presence of a microorganism in a sample of interest and a diagnostic kit for carrying out that method, i.e., one in which diagnostic results may be obtained within a few minutes to a few hours. More particularly, there exists an urgent need for a more rapid, inexpensive, reliable and user-friendly method and diagnostic kit for carrying out the method, which method and kit allow for the rapid diagnosis and treatment of UTI within the same rapid time period as discussed above.

SUMMARY OF THE INVENTION

The present invention unexpectedly and advantageously addresses the need for a highly sensitive and rapid method for detecting the presence of microorganism in a given sample within a few minutes to a few hours. More particularly, the present invention unexpectedly and advantageously addresses the need for a highly sensitive and rapid method for the diagnosis and treatment of UTI.

One aspect of the present invention is a method of detecting the presence of a microorganism in a sample. The method entails culturing the sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator. If a microorganism is present in the sample, the redox indicator advantageously and unexpectedly changes color within a rapid period of time, typically a few minutes to a few hours, depending on the number of bacteria present in the sample.

Another aspect of the present invention is a method of detecting the presence of a microorganism in a urine sample for diagnosing UTI. The method entails culturing the urine sample suspected of containing the microorganism in a growth medium containing a color-changing redox

indicator for a time sufficient to allow the microorganism to change the color of the redox indicator.

Yet another aspect of the present invention is a method of detecting and determining the cell count of a microorganism in a given sample. The method entails culturing the sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator, and observing the time required to change the color of the redox indicator to determine the cell count of the microorganism present in the sample. The more rapidly the redox indicator changes color, then the greater the number of microorganisms present in the sample.

Yet a further aspect of the present invention is a method of detecting and determining the cell count of a microorganism in a urine sample which allows for the diagnosis of UTI. The method calls for culturing the urine sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator, and then observing the time required to change the color of the redox indicator for the purpose of determining the cell count of the microorganism present in the urine. The determined cell count of the microorganism in the urine sample is then indicative of and diagnostic for the presence or absence of UTI.

The present invention further provides for a method of determining the antibiotic sensitivity or resistance of a microorganism in any given sample. The method entails culturing the sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator and an antibiotic, and observing the time required for the redox indicator to change color, or not change color at all, due to the inhibition of growth of a susceptible microorganism by the antibiotic.

Yet another aspect of the present invention is a method of detecting and identifying an unknown microorganism in any given sample. The method entails culturing the sample suspected of containing the microorganism in a selective growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator. The time required for the unknown microorganism to change the color of the redox indicator is then compared with that of a control sample of a known microorganism, or a number of known microorganisms of interest. Alternatively, the selective medium itself can be sufficient to provide the identity of the microorganism, since a change in the color of the redox indicator indicates the presence of the microorganism specific for the particular selective growth medium.

The present invention further advantageously provides for an embodiment of a diagnostic kit for detecting the presence of a microorganism in any given sample. The kit is comprised of a container containing a growth medium for supporting the growth of a microorganism, and a container containing a color-changing redox indicator. Alternatively, the growth medium and redox indicator may be pre-mixed and provided in the same container. The kits of the present invention can be readily utilized in a laboratory or a field setting for rapidly detecting the presence of a microorganism.

Yet another aspect of the diagnostic kit of the present invention is an additional container or containers containing an antibiotic or a number of antibiotics of interest for determining the antibiotic sensitivity or resistance of a microorganism in the test sample.

The present invention therefore unexpectedly and advantageously provides a method and diagnostic kit for carrying out the method for a more rapid detection of the presence of a microorganism in any given sample than is otherwise currently available.

The method and the kit of the present invention can be advantageously and unexpectedly utilized for the rapid detection of the total microbial load in any number of samples, including water, beverages, food, or any other material which can easily be homogenized and suspended in water. Other suitable samples which can be tested by way of the present invention include such biological fluids as urine, serum, whole blood, sputum, throat and fecal specimens, as well as vaginal, pleural, or spinal fluid, as well as other potentially infected bodily fluids. Thus, the method and kit of the present invention are useful in any number of a broad variety of applications which call for the rapid detection of microbial contamination. For example, public undertakings, such as municipal water supply systems, and food or pharmaceutical industries will benefit by making use of the method and kit of the present invention. For example, by way of the present invention, it will be possible to detect *Shigella dysenteriae* type 1 infection in developing countries. It will also be possible to detect the presence of *Salmonella, Campylobacter, Listeria* and *E. coli*, as well as other microorganisms, in food and beverages with the methods and kits of the present invention.

More particularly, the method and kit of the present invention are useful for the unexpectedly rapid diagnosis and treatment of UTI. As opposed to the 24 to 48 hours typically required to obtain provisional results by present conventional methods, the method and kit of the present invention can provide information on the presence of a microorganism, the identity, the number of microorganisms present in a sample of urine, and the sensitivity or the resistance of these microorganisms to commonly used antibiotics within a very short period of time, advantageously and unexpectedly within about 30 minutes to about 2 hours. This is advantageously and surprisingly much more rapid than other currently available techniques.

Furthermore, the method and kit of the present invention are advantageously useful for the rapid determination of the antibiotic sensitivity of a microorganism causative of UTI. By way of the present invention, antibiotic therapy, specific to the organism found in the urine by the novel methods and kits of this invention, can be undertaken which might rapidly improve the patient's condition. In life-threatening situations, a rapid determination of the suitable antibiotic regimen for treating UTI may well mean the difference between life and death.

The present invention is also particularly suitable for the diagnosis and treatment of outpatient and "subacute" UTI, as well as other routine clinical infections. The present invention is therefore particularly advantageous with respect to its widespread use for the diagnosis of routine clinical infections.

The kit of the present invention is advantageously small, portable, lightweight, reasonably priced and user-friendly, since the results are facilely determined by visual inspection without requiring any mechanical apparatus.

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims.

DETAILED DESCRIPTION OF THE INVENTION

As described in detail hereinafter, the present invention advantageously and unexpectedly provides for a method and a diagnostic kit for detecting the presence of a microorganism in any given sample within a rapid period of time. Furthermore, the cell count, antibiotic sensitivity or resistance, and identity of the unknown microorganism in a given sample can also be advantageously

determined by way of the method and kit of the present invention within an unexpectedly short period of time.

More particularly, the presence, cell count, antibiotic sensitivity or resistance, and identity of an unknown microorganism in a urine sample can be more rapidly determined by way of the method and kit of the present invention as an important aid in the diagnosis and appropriate treatment of UTI. This diagnosis of UTI is advantageously suitable for either outpatient or inpatient situations, as well as for the diagnosis and treatment of subacute UTI.

The method of the present invention advantageously provides for more rapidly detecting the presence of a microorganism in any given sample. The method is carried out by culturing the sample suspected of containing the microorganism in a suitable growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator. The redox indicator changes color in response to the chemical reduction of the growth medium by the microorganism.

A redox indicator provides a useful scale of measuring the degree of anaerobiosis, and is a measure of the tendency of a solution to give or take-up electrons, which is expressed in units of electrical potential difference. See Jacob, H.E., "Redox Potential" in J.R. Novis and D.W. Ribbons (Ed) *Methods in Microbiology*, 2, Academic Press, N.Y., 99-123 (1970), which is incorporated herein by reference. The redox indicator utilized in the present invention provides a useful means of determining oxidation-reduction potential in a biological system by changing from one color to another or to a colorless state, and vice versa. See *Dictionary of Microbiology and Molecular Biology*, 2nd Ed., pp. 746-747 by Paul Singleton & Diana Sainsbury, John Wiley & Sons, New York, NY, (1987), which is incorporated herein by reference.

A particularly advantageous aspect of the present invention is that any sample suspected of containing a microorganism, which sample can be readily homogenized and suspended in water, can be screened for the presence of the microorganism by way of the methods and kits of the present invention.

With regard to carrying out the method of the present invention, any medium which is capable of sustaining the growth of a microorganism is considered suitable for practice in the methods and kits of the present invention. Non-limiting examples of such basal media include Oxford, Hektoen enteric, xylose-lysine-deoxycholate, Sabouraud broth (Emmon's modification), mannitol salt, brilliant green, EMB, cetrimide, potato malt and KF Streptococcus agars, among others, along with improved modifications thereof, which are disclosed in U.S. patent application Serial No. ____, filed _____. Also see Examples 4 and 5 herein. The improved media formulations particularly suitable for practice of the present invention are disclosed in Example 5. Most particularly suitable is a modified Trypticase soy broth (TSB) medium.

To detect the presence of a microorganism in a test sample which has been inoculated into the growth medium, a color-changing redox indicator is incorporated into the growth medium. After a sufficient amount of time allowing for the growth or metabolic activity of the microorganism, the color of the redox indicator changes. By way of the present invention, the time required for the redox indicator to change color is a short period, from within a few minutes to a few hours, typically about 30 minutes to about 2 hours. The more microorganisms present, the shorter the time required for the redox indicator to change color. This color change thus then rapidly indicates the presence of a microorganism.

It is contemplated that any redox indicator which is not toxic to the growth of a bacterial or fungal cell is suitable for practice in the present invention. Non-

limiting examples of such suitable redox indicators are methylene blue, toluidine blue, resorufin, tetrazolium, phenol red, bromocresol purple, indigo trisulfonate, and 1,5-anthraquinone sulfate. A particularly suitable redox indicator for use in the method and kit of the present invention is Alamar Blue (AB) from Alamar Biosciences, Inc., Sacramento, California. AB changes color from blue to red, and eventually to pale yellow, in response to the chemical reduction of growth medium which results from microbial growth or metabolic activity. The present invention takes advantage of the rate of color change of the redox indicator being directly proportional to the degree of metabolic activity and/or number of microbial cells present in the test sample.

Any number of samples of interest can be screened for the presence of microbial contamination by way of the present invention. Non-limiting examples include water, beverages, food, or any other material which can be readily homogenized and suspended in water. Other suitable samples which can be tested by way of the present invention include biological fluids such as urine, serum, whole blood, sputum, throat and fecal specimens, and vaginal, pleural, and spinal fluids. In such fashion then, the present invention is suitable for detecting the presence of a clinical infection, routine or otherwise, by screening a bodily fluid of interest. This includes either an inpatient or outpatient setting.

By way of the method and diagnostic kit of the present invention, it is contemplated that the presence of any microorganism can be detected in a given sample, including bacterial as well as fungal microorganisms. Of particular interest are those microorganisms, which have been identified above, as well as others, responsible for causing UTI.

The method of the present invention may be carried out as follows. A volume of the test sample suspected of containing a microorganism is mixed with an equal volume

of double strength growth medium contained in an appropriate reaction vessel. A pre-sterilized amount of the redox indicator is added to give a final concentration of from about 5% to about 15%, preferably about 10% (v/v). The mixture is incubated at an appropriate temperature, for example, about 35°C-37°C. If an appropriate heating mechanism is unavailable, the samples may be held close to the body. The tubes are examined periodically, for example, about every 10 to 15 minutes, for detecting or observing the color change of the redox indicator in response to the presence of a microorganism.

For a patient suspected of having a UTI, a urine sample can be collected and tested by way of the present invention for permitting a rapid diagnosis of UTI. A sample of the urine can be mixed with the growth medium and redox indicator, and incubated, as described above. If a sufficient number of organisms are present in the urine sample, then the redox indicator advantageously and unexpectedly changes color within a few minutes to a few hours, typically about 30 minutes to about 2 hours. This advantageously and unexpectedly provides for the rapid detection and diagnosis of UTI. Typically, at least 10^5 organisms/ml are indicative of UTI, although the present method can rapidly and advantageously determine the presence of even fewer organisms/ml of urine in a patient with UTI.

A particular UTI of growing interest and concern is Acute Urethral Syndrome (AUS), which is generally observed in young (under 45), sexually active persons, typically female. In such patients, there may be 10^2 or even fewer organisms/ml of urine. While the redox indicator may take slightly longer to change color, e.g., about four to about six hours, the present invention is considered advantageously suitable for aiding in the diagnosis of AUS.

Furthermore, by way of the present invention, one can advantageously determine the antibiotic sensitivity

or resistance of a microorganism present in the test sample more rapidly as compared with other conventional methods. This can be readily accomplished by incorporating an antibiotic of interest into the growth medium containing the redox indicator. A suitable amount of the test sample suspected of containing the microorganism is inoculated into this medium, simultaneously along with the sample incubated in the medium containing the redox indicator alone as a control. Typically, the concentrations of antibiotic utilized range from one dilution below the accepted minimum inhibitory concentration (MIC), the MIC, and one dilution above the MIC. These concentrations can be routinely determined by one of skill in the art. The appropriate amount of antibiotic is mixed with the growth medium containing the redox indicator and then inoculated with the test sample suspected of carrying a microorganism. Depending upon the number of microorganisms present in the sample, the results determining antibiotic sensitivity of the microorganisms are available within a few minutes to a few hours.

In this fashion, the inhibitory effect, if present, of the antibiotic on the metabolic growth of the microorganism can be compared with that of the microorganism inoculated into the growth medium containing the redox indicator alone. By comparing the times required for changing the color of the redox indicators of the respective cultures, one can readily ascertain whether the antibiotic possesses an inhibitory effect on the growth or metabolism of the microorganism present in the sample.

If the microorganism is sensitive to the antibiotic, the color change observed for the redox indicator will occur more slowly, if at all, as compared to the control.

The advantages of a rapid determination of antibiotic sensitivity of a microorganism are readily apparent, particularly with regard to treating a patient infected with that microorganism.

Any antibiotic of interest can be utilized in the method and diagnostic kit of the present invention. With regard to antibiotics of particular interest for treating UTI, trimethoprim, trimethoprim-sulfamethoxasol (TMP-SMX), amoxicillin and broad spectrum cephalosporins (Cipro and Duricef) are particularly suitable. Other suitable, but non-limiting, antibiotics include cephalothin, chloramphenicol, gentamycin, kanamycin, polymyxin and tetracycline. These also include antibiotics prescribed for inpatients or outpatients.

Typically in current medical practice, trimethoprim has most frequently been prescribed by physicians in ambulatory cases even if the culture results are not available. Trimethoprim has proven effective against a wide range of microorganisms including *E. coli*. Third generation cephalosporins have also frequently been prescribed in cases in which symptoms of UTI are present, but cultures are generally either not carried out, or the results are not available to the physician prior to antibiotic therapy.

Accordingly, by way of the present invention, the physician advantageously and unexpectedly can make a more informed decision regarding the appropriate antibiotic to be administered to the patient in need thereof.

The present invention also encompasses within its scope antibiotics which are presently unavailable, but rather will be later developed.

By way of the present invention, one can further advantageously determine the total microbial count of any given test sample. As a general rule, the more microorganisms present in the test sample, the more rapidly the redox indicator changes color. For instance, the redox indicator may change color in 60 minutes when 10^6 microorganisms/ml are present, as compared with a color change in 90 minutes when 10^5 microorganisms/ml are present. One of skill in the art can readily compare the time required to change the color indicator present in the growth medi-

um by a microorganism in the test sample with that of a standardized table or graph showing the time change of the redox indicator with respect to the known number of microorganisms/ml.

For any given microorganism, such a standard graph or curve of time for color change of redox indicator vs. number of microorganisms/ml can be readily carried out by titrating a number of ten-fold serial dilutions of the organism, e.g. 10^2 through 10^6/ml, inoculating the serial dilutions into growth medium containing redox indicator, and observing the varying times for the redox indicator to change color as a function of cell concentration.

For example, such a cell count profile may read as follows:

Number of Microorganisms	Time for Redox Indicator to Change Color
10^2/ml	4 hours
10^3/ml	2 hours
10^4/ml	1 hours
10^5/ml	30 minutes
10^6/ml	15 minutes

One can readily compare the time required for the microorganism of unknown concentration in the test sample to change the color of the redox indicator with that of the standard or control results for known concentrations to ascertain the number of microorganisms/ml in the test sample.

By way of the present invention, one may also identify the microorganism of interest present in the sample. By incubating an aliquot of the sample containing the microorganism with the growth medium and redox indicator as described above, one observes a color change within a given period of time. By comparing the time required for the microorganism to change the color of the redox indicator with that of a standardized table of known microor-

ganisms, one can readily identify the microorganism present in the test sample.

Alternatively, one may culture the unknown microorganism in a selective growth medium which specifically favors or selects for the growth of a particular microorganism or group of microorganisms. Such media are disclosed herein, and others are readily known to one of skill in the art. By incorporating a suitable redox indicator into the selective growth medium as described above, one can readily observe whether the indicator changes color. Such a color change of the medium specific for a particular microorganism is indicative of the presence of that microorganism in the sample of interest. Depending upon the number of organisms present in the sample, one can rapidly identify, within about a few minutes to about a few hours, the particular microorganism present in the sample.

Yet another advantageous aspect of the present invention is a diagnostic kit suitable for commercial sale for detecting the presence of a microorganism in any given sample. The kit is comprised of a container containing a suitable growth medium for a microorganism, and a container containing a color changing redox indicator. Alternatively, the growth medium and redox indicators may be provided pre-mixed in one container. The diagnostic kit of the present invention may further contain an antibiotic or number of antibiotics of interest which enable the determination of the antibiotic sensitivity or resistance of the microorganism in the given test sample. The kits of the present invention thus allow for the simultaneous determination of the presence, identity, and antibiotic sensitivity of a microorganism in a given test sample.

The kits of the present invention are advantageously small, portable, light-weight, reasonably priced, and user-friendly. The results are rapidly determinable by visual inspection without the aid of any device. No

special training or background is required to utilize the kit of the present invention. The kit may be readily utilized in either a clinical or field setting. For example, a small amount of sample is added to a container or tube containing the mixture of growth medium and redox indicator. The tube is incubated at an appropriate temperature, e.g. about 35°C or held close to the body (in a safe plastic bag). The tube is then observed periodically, for example, every 15 to 30 minutes, to detect a color change.

The following examples are for the purpose of illustrating the present invention and should not be construed as limiting in any respect.

EXAMPLES

Example 1

Determination of Time for Color Change Per Cell Concentration

One strain each of *E. coli* (ATCC 8739) and *E. faecalis* (ATCC 19433) were selected. Serial 10-fold dilutions were prepared in sterile distilled water. One milliliter of the suspension containing a known amount of the bacterial cell was mixed with an equal amount of a double strength TSB contained in a sterile test tube. A presterilized AB solution was added to give a final concentration of 10% (v/v). The mixture was incubated at 35°C and the tubes were examined every 10 minutes for the color change. It was observed that in the case of *E. coli*, the medium's color changed from purple to red in 1 hour if the initial bacterial count was 10^6 CFU/ml. If the initial counts were 10^5, 10^4, or 10^3/ml, the time required for the color change was 90, 120 and 150 minutes, respectively. Results were obtained somewhat faster in the case of *S. faecalis*. The suspension containing 10^6 cell/ml became red within 45 minutes. Those contain-

ing 10^5, 10^4, 10^3 cell/ml changed from blue to red in 60, 90, and 120 minutes, respectively.

Example 2

Prophetic Determination of Total Microbial Count

The basal medium will be prepared by dissolving 17 g tryptone (pancreatic digest of casein), 5 g yeast extract, 3 g soytone (papiac digest of soybean meal), 5.0 g lactose, 5 g sodium chloride, and 2.5 g dipotassium phosphate in 500 ml distilled water and adjusting the pH to 7.2. One ml of this medium will be dispensed into 10 cm x 1.5 cm screw-capped test tubes and sterilized by autoclaving at 120°C for 15 minutes. After the medium has cooled down to room temperature, 0.2 ml of the Alamar Blue will be aseptically added into each tube. The Alamar Blue is presterilized by filtration. The mixture named Total Count Medium (TCM) will be stored at 4°C.

Initial studies will be performed using cultures of the following reference strains obtained from the American Type Culture Collection (ATCC):

Escherichia coli	ATCC 8739
Klebsiella pneumonia	ATCC 9997
Proteus mirabilis	ATCC 43071
Streptococcus faecalis	ATCC 19433
Staphylococcus saprophyticus	ATCC 49453

Cultures of the test strains will be grown for 24 hours at 35°C on blood agar. The bacterial growth will be harvested and the cell density determined calorimetrically followed by viable counts using the dilution method and culture on TSB. Serial ten-fold dilutions will be prepared in sterile distilled water to obtain 10^6, 10^5, 10^4, 10^3, or 10^2 CFU/ml. One ml of the suspension will be mixed with the TCM and incubated at 35°C. A tube containing the TCM plus 1 ml of distilled water will be included as a control. The tubes will be observed every 15 minutes for change in the color from blue to red and eventually to pale yellow. Results will be tabulated to

show the microbial cell density and the time required for the color change.

The experiment will be repeated using urine samples collected from healthy volunteers whose urine has been found to contain no bacteria. Instead of using sterile distilled water, the inoculum will be prepared in the urine sample and one ml amounts will be mixed with the TCM. Incubation and observations will be made as described above. The range of strain-to-strain variation will be studied by testing three strains of the aforementioned species. This part of the experiment will elucidate the effect of urine and its constituents on the redox reaction.

Example 3

Prophetic Determination of Antibiotic Sensitivity

Stock solutions of the test antibiotics will be prepared in buffered saline and stored at 4°C. The basal medium for this purpose will be prepared by dissolving 21 g of Bacto Mueller Hinton Broth (Difco Laboratories, Detroit, MI) supplemented with 0.5% yeast extract in 500 ml of distilled water (pH 7.3). One ml of this medium will be dispensed into 10 cm x 1.5 cm screw-capped test tubes and sterilized by autoclaving at 120°C for 15 minutes. After the tubes have cooled down, a 0.2 ml aliquot of antibiotic will be aseptically added to each tube and known amounts of test antibiotics will be added to give a final concentration equal to the MIC of the individual antibiotics. Two additional concentrations, one a dilution below the MIC and the other a dilution above the MIC will be used. The decision to include this range in the antibiotic sensitivity testing is based on the generally accepted principle that a deviation from the expected MIC value by one dilution in either direction is insignificant. The mixture named antibiotic sensitivity medium (ASM) will be inoculated with the test organisms as described before. The initial study will be done with

microbial suspensions prepared in distilled water. The experiment will be repeated using artificially inoculated urine samples from healthy donors. The mixture will be incubated at 35°C and color change, if any, recorded every 15 minutes. Comparison of results from bacterial suspensions prepared in distilled water and human urine will be analyzed to determine the effect of urine on the redox reaction and the color change.

Example 4

Preparation of Other Commercial Formulations

The following are examples of agar formulations suitable for carrying out the present invention.

a. Mannitol salt agar (BBL) (widely used in medium for *Staphylococcus aureus*):

Mannitol salt agar (11.1 g) was suspended in 100 ml of deionized water and heated to dissolve completely. It was sterilized in the autoclave for 15 min. and 15 lb. pressure, cooled at 48°C and poured into petri dishes. The final pH = 7.4.

b. Brilliant green agar (BBL) (selective medium for *Salmonella sp.*):

Brilliant green agar (5.8 g) was suspended in 100 ml of deionized water and heated to dissolve completely. It was sterilized in the autoclave for 15 min. at 15 lb. pressure, cooled at 48°C and poured into petri dishes. The final pH = 6.9.

c. EMB agar (BBL) (widely used medium for Gram negative organisms.:

EMB agar (3.6 g) was suspended in 100 ml of deionized water and heated to dissolve completely. It was sterilized for 15 min. at 15 lb. pressure, cooled at 48°C and poured into petri dishes. The final pH = 7.1.

d. Cetrimide agar (Difco) (selective for *Pseudomonas aeruginosa*):

Cetrimide agar (4.53 g) was suspended in 100 ml of deionized water and heated to dissolve completely, then

added 1.0 ml of glycerol. It was sterilized for 15 min. at 15 lb. pressure, cooled at 48°C and poured into petri dishes. The final pH = 7.2.

e. Potato malt agar (Difco) (widely used for fungi):

Potato malt agar (10.5 g) was suspended in 100 ml of deionized water and heated to dissolve completely. It was sterilized in the autoclave for 15 min. at 15 lb. pressure, cooled at 48°C and poured into petri dishes. The final pH = 7.4.

f. KF Streptococcus agar (Difco), (selective medium for enterococcus).

KF Streptococcus agar (5.64 g) was suspended in 100 ml of deionized water and heated to dissolve completely. It should not be autoclaved. After cooling to 48°C, 1.0 ml of triphenyl- tetrazolium chloride (TTC) was added to 100 ml of media. The final pH = 7.2.

Example 5

Modifications of Commercial Formulations

The following are examples of modified broth formulations suitable for carrying out the present invention.

Mannitol Salt Medium

Ingredient		Weight/Volume
Mannitol	1.0 g	1.00%
Peptone	1.0 g	1.00%
Magnesium sulfate	0.1 g	.10%
Phenol red	0.0025 g	0.0025%
Sodium chloride	0.75 g	0.75%
Deionized water (pH 7.2)	100 ml	

All the ingredients (mannitol, peptone, magnesium sulfate, phenol red and sodium chloride) were weighed in an analytical balance and dissolved in 100 ml of deion-

ized water by heating and stirring. The pH was adjusted to 7.2. The medium was heated to boiling and was sterilized for 15 min. at 15 lb. pressure, cooled at 48°C and poured in increments of 4 ml, 6 ml and 8 ml of media in suitable vessels.

Brilliant Green Medium

Ingredient		Weight/Volume
Peptone	1.0 g	1.00%
Lactose	1.0 g	1.00%
Magnesium sulfate	0.1 g	0.1 g
Brilliant green	0.00125 g	0.00125%
Phenol red	0.08 g	0.08%
Calcium carbonate	0.05 g	0.05%
Deionized water	100 ml	

pH adjusted to 6.9

All the ingredients including peptone, lactose, magnesium sulfate, media, brilliant green, phenol red, calcium carbonate were dissolved in 100 ml of deionized water by heating. The mixture was cooled and adjusted to a pH of 6.9. The mixture was then sterilized for 15 min. at 15 lb. pressure, cooled to 48°C and poured in increments of 4 ml, 6 ml and 8 ml of media in suitable vessels.

EMB Medium

Ingredient		Weight/Volume
Peptone	1.0 g	1.00%
Lactose	1.0 g	1.00%
Eosin dye	0.04 g	0.04%
Methylene blue	0.0065 g	0.0065%
Magnesium sulfate	0.1 g	0.10%
Deionized water	100 ml	

pH adjusted to 7.2

Peptone, lactose, eosin dye, methylene blue, and magnesium sulfate were weighed and dissolved in 100 ml of deionized water with heating and stirring. The pH was adjusted to 7.2. It was sterilized for 15 min. at 15 lb. pressure. The medium was cooled to 48°C and poured in increments of 4 ml, 6 ml and 8 ml of media in the designated culture containers.

Cetrimide Base medium

Ingredient Weight/Volume		
Cetrimede	0.03 g	0.03%
Peptone	2.0 g	2.00%
Magnesium sulfate	0.1 g	0.10%
Calcium carbonate	0.1 g	0.10%
Glycerol	1.0 ml	1.00%
Deionized water	100 ml	

pH adjusted to 7.2

Cetrimide, peptone, magnesium sulfate, and calcium carbonate were weighed accurately and dissolved in 100 ml of deionized water and 1.0 ml of glycerol with heating and stirring. The pH was adjusted to 7.2. The medium was heated to boiling. It was sterilized for 15 min. at

15 lb. pressure. The medium was cooled to 48°C and poured in increments of 4 ml, 6 ml and 8 ml of media.

Potato Malt Medium

Ingredient		Weight/Volume
Potato starch	3.0 g	3.00%
Malt	3.0 g	3.00%
Dextrose	6.0 g	6.00%
Magnesium sulfate	0.1 g	0.10%
Deionized water	100 ml	

pH was adjusted to 7.4

Potato starch, malt, dextrose, and magnesium sulfate were weighed accurately and dissolved in 100 ml of deionized water with heating and stirring. The pH was adjusted to 7.4. The medium was heated to boiling and was sterilized for 15 min. at 15 lb. pressure. The medium was cooled to 48°C and poured in increments of 4 ml, 6 ml and 8 ml of media.

Streptococcus Medium

Ingredient Weight/Volume		
Soytone	1.0 g	1.00%
Yeast extract	1.0 g	1.00%
Lactose	0.1 g	0.10%
Malt extract	0.2 g	0.20%
Magnesium sulfate	0.1 g	0.10%
Sodium chloride	0.50 g	0.50%
Potassium phosphate monobasic	0.5 g	0.50%
Sodium azide	0.048 g	0.048%
Bromocresol purple	0.0015 g	0.0015%
Deionized water	100 ml	

pH adjusted to 7.2

Triphenyl tetrazolium
chloride 1.0 ml

All the ingredients were weighed accurately and dissolved in deionized water, pH adjusted to 7.2 and heated to boiling. The medium was cooled to 48°C and 1.0 ml of 1% triphenyl- tetrazolium chloride (TTC) was added. The medium was poured into the designated test tubes in 4 ml, 6 ml and 8 ml quantities.

In summary, the present invention advantageously and unexpectedly provides a method and diagnostic kit for the rapid detection of a microorganism in any given sample or specimen. The antibiotic sensitivity, total cell count of the microorganism in the sample, and the identity of the microorganism can all be rapidly determined by way of the present invention. More specifically, the present invention provides for a method and diagnostic kit that permits the detection of UTI in less than 1/10th of the time required by currently available conventional methods, while also providing valuable information on the antibiotic sensitivity of the infectious microorganism, which allows for a more accurate and informed treatment of UTI in a patient by a physician.

It is to be understood that the foregoing detailed description is merely illustrative and is not to be taken as limiting upon the scope of the invention, which is defined solely by the appended claims and their equivalents. Various changes and modifications will be apparent to those of skill in the art. Such changes and modifications may be made without departing from the spirit and scope thereof of the present invention.

We claim:

1. A method of rapidly detecting the presence of a microorganism in a sample comprising culturing the sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator, which redox indicator rapidly changes color in response to the presence of the microorganism.

2. The method of claim 1 wherein the sample is selected from the group consisting of water, beverage and food.

3. The method of claim 1 wherein the sample is selected from the group consisting of serum, whole blood, sputum, throat and fecal specimens, vaginal, pleural fluid, and spinal fluid.

4. The method of claim 1 wherein the redox indicator is selected from the group consisting of tetrazolium, resorufin, phenol red, bromocresol purple, methylene blue, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and alamar blue.

5. The method of claim 4 wherein the redox indicator is alamar blue.

6. The method of claim 5 wherein the alamar blue changes color from blue to red.

7. The method of claim 1 wherein the growth medium is a modified trypticase soy broth.

8. The method of claim 1 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

9. A method of rapidly detecting the presence of a microorganism in a urine sample for diagnosing urinary tract infection which comprises culturing the urine sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator, which redox indicator rapidly changes color in response to the presence of the microorganism.

10. The method of claim 9 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

11. The method of claim 10 wherein the redox indicator is alamar blue.

12. The method of claim 11 wherein the alamar blue changes color from blue to red.

13. The method of claim 9 wherein the growth medium is a modified trypticase soy broth.

14. The method of claim 9 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

15. A method of rapidly detecting the presence and determining the cell count of a microorganism in a sample comprising the steps of culturing the sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator, and observing the time required to change the color of the redox indicator for the purpose of determining the cell count of the microorganism present in the sample.

16. The method of claim 15 wherein the sample is selected from the group consisting of water, beverage and food.

17. The method of claim 15 wherein the sample is selected from the group consisting of serum, whole blood, sputum, throat and fecal specimens, and vaginal, pleural, and spinal fluids.

18. The method of claim 15 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

19. The method of claim 18 wherein the redox indicator is alamar blue.

20. The method of claim 19 wherein the alamar blue changes color from blue to red.

21. The method of claim 15 wherein the growth medium is a modified trypticase soy broth.

22. The method of claim 15 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

23. A method of rapidly detecting the presence and determining the cell count of a microorganism in a urine sample for diagnosing urinary tract infection comprising the steps of culturing the urine sample suspected of containing the microorganism in a growth medium containing a color-changing redox indicator for a time sufficient to allow the microorganism to change the color of the redox indicator, and observing the time required to change the color of the redox indicator for the purpose

of determining the cell count of the microorganism present in the urine, said cell count being diagnostic for the presence or absence of urinary tract infection.

24. The method of claim 23 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

25. The method of claim 24 wherein the redox indicator is alamar blue.

26. The method of claim 25 wherein the alamar blue changes color from blue to red.

27. The method of claim 23 wherein the growth medium is a modified trypticase soy broth.

28. The method of claim 23 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

29. A method of rapidly determining the antibiotic sensitivity or resistance of a microorganism in a sample which comprises culturing the sample suspected of containing the microorganism in a medium containing a color-changing redox indicator and an antibiotic, and observing the time required to change the color of the redox indicator.

30. The method of claim 29 wherein the sample is selected from the group consisting of water, beverage and food.

31. The method of claim 29 wherein the sample is selected from the group consisting of urine, serum, whole

blood, sputum, throat and fecal specimens, vaginal, pleural, and spinal fluids.

32. The method of claim 29 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

33. The method of claim 32 wherein the redox indicator is alamar blue.

34. The method of claim 33 wherein the alamar blue changes color from blue to red.

35. The method of claim 29 wherein the growth medium is a modified trypticase soy broth.

36. The method of claim 29 wherein the growth medium is a modified Mueller Hinton broth.

37. The method of claim 29 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

38. The method of claim 29 wherein the antibiotic is selected from the group consisting of trimethoprim, amoxicillin, cephalosporin, cephalothin, chloramphenicol, gentamycin, kanamycin, polymyxin, and tetracycline.

39. A method of rapidly detecting and identifying an unknown microorganism in a sample which comprises culturing the sample suspected of containing the microorganism in a selective growth medium containing a color-changing redox indicator for a time sufficient to change the color of the redox indicator, said color change being

indicative of the presence of a microorganism specific for said growth medium.

40. The method of claim 39 wherein the sample is selected from the group consisting of water, beverage and food.

41. The method of claim 39 wherein the sample is selected from the group consisting of urine, serum, whole blood, sputum, throat and fecal specimens, vaginal, pleural, and spinal fluids.

42. The method of claim 41 wherein the sample is urine.

43. The method of claim 39 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

44. The method of claim 43 wherein the redox indicator is alamar blue.

45. The method of claim 44 wherein the alamar blue changes color from blue to red.

46. The method of claim 39 wherein the growth medium is a modified trypticase soy broth.

47. The method of claim 39 wherein the redox indicator changes color within about 15 minutes to about 2 hours.

48. A diagnostic kit for rapidly detecting the presence of a microorganism in a sample which comprising:

a container containing a growth medium; and

a container containing a color-changing redox indicator.

49. The diagnostic kit of claim 48 wherein the growth medium is selected from the group consisting of a modified trypticase soy broth, Oxford, Hektoen enteric, xylose-lysine-deoxycholate, Sabouraud broth, mannitol salt, brilliant green, EMB, cetrimide, potato malt and KF Streptococcus.

50. The diagnostic kit of claim 48 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

51. A diagnostic kit for rapidly detecting the presence of a microorganism in a sample which comprises:

a container containing a growth medium;

a container containing a color-changing redox indicator; and

a container containing an antibiotic.

52. The diagnostic kit of claim 51 wherein the growth medium is selected from the group consisting of a modified trypticase soy broth, Oxford, Hektoen enteric, xylose-lysine-deoxycholate, Sabouraud broth, mannitol salt, brilliant green, EMB, cetrimide, potato malt and KF Streptococcus.

53. The diagnostic kit of claim 51 wherein the redox indicator is selected from the group consisting of phenol red, bromocresol purple, alamar blue, tetrazolium, resorufin, indigo trisulfonate, 1,5-anthraquinone sulfate, toluidine blue, and methylene blue.

54. The diagnostic kit of claim 51 wherein the antibiotic is selected from the group consisting of trimethoprim, amoxicillin, cephalosporin, cephalothin, chloramphenicol, gentamycin, kanamycin, polymyxin, and tetracycline.

55. A diagnostic kit for rapidly detecting the presence of a microorganism in a sample which comprises:

a container containing a mixture of growth medium and a redox indicator.

56. The diagnostic kit of claim 55 wherein the mixture further comprises an antibiotic.

57. The diagnostic kit of claim 55 which further comprises a container containing an antibiotic.

58. The diagnostic kit of claim 57 wherein the antibiotic is selected from the group consisting of trimethoprim, amoxicillin, cephalosporin, cephalothin, chloramphenicol, gentamycin, kanamycin, polymyxin, and tetracycline.

59. The diagnostic kit of claim 56 wherein the antibiotic is selected from the group consisting of trimethoprim, amoxicillin, cephalosporin, cephalothin, chloramphenicol, gentamycin, kanamycin, polymyxin, and tetracycline.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US96/03223

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12Q 1/04, 1/16, 1/18; G01N 21/64, 21/76
US CL :435/32, 34, 35, 810; 250/461.2; 436/172, 800
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/32, 34, 35, 810; 250/461.2; 436/172, 800

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, MEDLINE, DERWENT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	EP, A3, 0 322 591 (ABBOTT LABORATORIES) 05 July 1989, see pages 3, 4, and 9-10.	1-4, 9-10, 15-18, 23, 24, 29-32, 37-38, 48, 50-51, 53-59 --- 5-8, 11-14, 19-22, 25-28, 33-36, 39-47, 49, 52
Y	US, A, 5,336,600 (MONGET) 09 August 1994, see columns 4, 7, 9, 12 and 13.	5-8, 11-14, 19-22, 25-28, 33-36, 39-47, 49, 52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 APRIL 1996	30 APR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/03223

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	US, A, 5,164,301 (THOMPSON ET AL.) 17 November 1992, see columns 4, 6, 7, 10, 11, and 14-16.	39
A	EP, A1, 0 625 581 (BIOTEST AG) 23 November 1994, see entire document.	1-59